Expanded View Figures

Figure EV1. mH2A1.2 is predominantly expression in the developing brain and neural progenitor cells.

A qRT–PCR analysis shows that exogenous expression of mH2A1 with mH2A2. $n = 3$ mice, independent replicates.
B qRT–PCR analysis shows that exogenous expression of mH2A1.1 with mH2A1.2 $n = 3$ mice, independent replicates.
C The temporal expression (E13, E15, E17, and P0) of mH2A1.1 and mH2A1.2 mRNAs by qRT–PCR in the developing cortex.
D mH2A1.2 is co-labeled with PAX6 and TUJ1 in neural progenitor cells and neurons cultured in vitro. Scale bar represents 50 μm.
E Western blot analysis shows that exogenous Flag-mH2A is efficiently reduced in mH2A1.2-shRNA-transfected 293 cells.
F Graph shows that the amount of mH2A is obviously decreased in mH2A1.2-shRNA-transfected 293 cells. $n = 5$ samples, independent replicates.
G Immunostaining images show that endogenous mH2A1.2 is obviously reduced in mH2A1.2-shRNA-electroporated cells. Hatched lines are cell boundaries.

Date information: Representative images from at least three independent experiments. Error bars represent means ± S.E.M.; two-tailed unpaired *t*-test, $^{**}P < 0.01$ or $^{***}P < 0.001$. n.s., not significant.

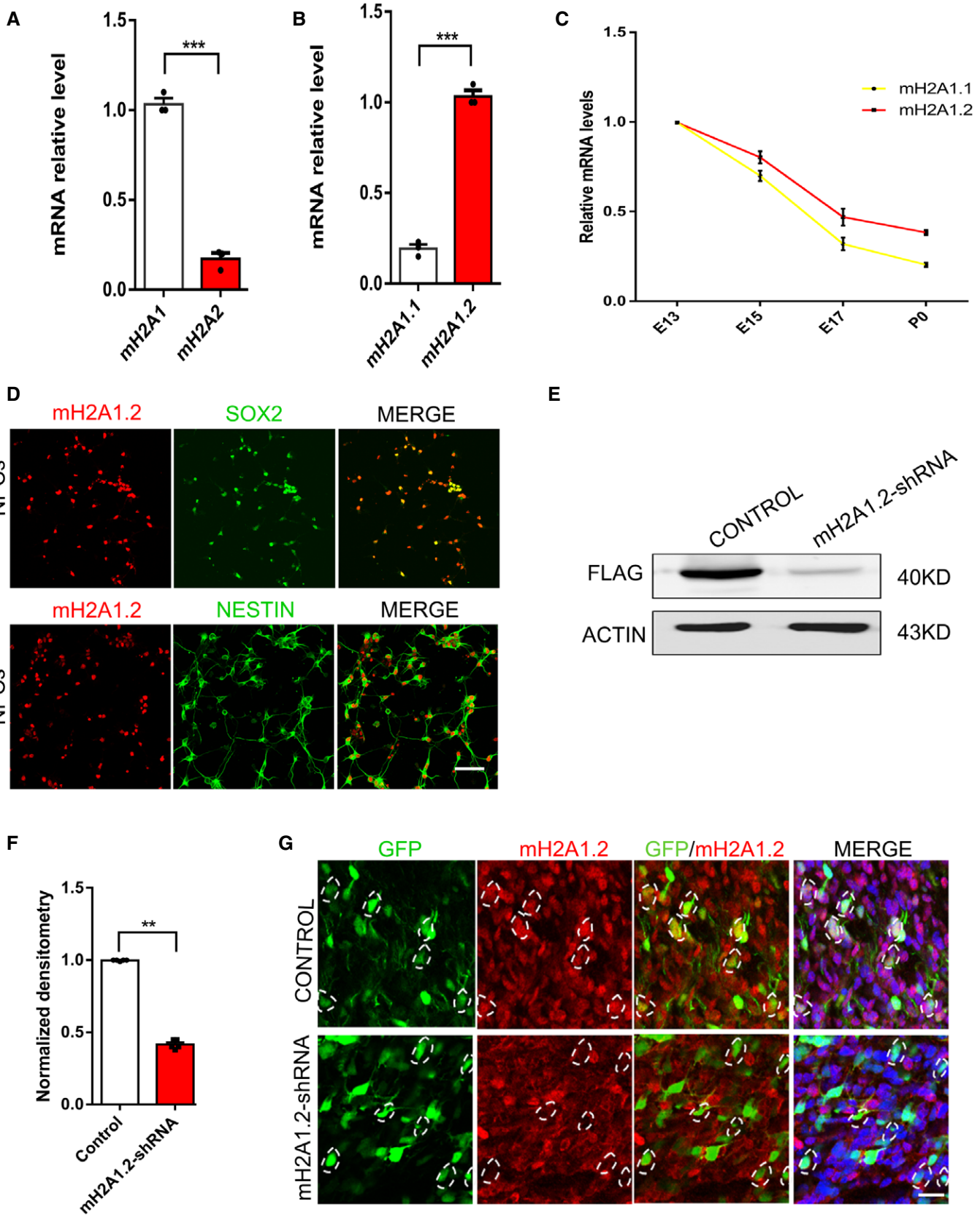
A
mRNA relative level
1.5
1.0
0.5
0.0
***
mH2A1
mH2A2
B
mRNA relative level
1.5
1.0
0.5
0.0
***
mH2A1.1
mH2A1.2
C
Relative mRNA levels
1.5
1.0
0.5
0.0
mH2A1.1
mH2A1.2
E13
E15
E17
P0
D
mH2A1.2
SOX2
MERGE
NPCs
mH2A1.2
NESTIN
MERGE
NPCs
E
CONTROL
mH2A1.2-shRNA
FLAG
40KD
ACTIN
43KD
F
Normalized densitometry
1.5
1.0
0.5
0.0
**
Control
mH2A1.2-shRNA
G
GFP
mH2A1.2
GFP/mH2A1.2
MERGE
CONTROL
mH2A1.2-shRNA

Figure EV1.

Figure EV2. mH2A1.2 regulates the proliferation of neural progenitor cells.

A Counted the number of GFP^+ cells in the same size area after E16 harvesting of the brain and found no significant differences. $n = 6$ mice, independent replicates.
B Expression of mH2A1.2 at the WT and KO mRNA levels was analyzed by qRT–PCR. $n = 5$ mice, independent replicates.
C Expression of mH2A1.1 and mH2A1.2 at the WT and KO mRNA levels was analyzed by qRT–PCR. $n = 5$ mice, independent replicates.
D Immunostaining shows that the expression of mH2A1.2 is depleted in the E16.5 KO mice brains. Scale bar represents 50 μm.
E Mouse embryos were electroporated in utero with control, mH2A1.2-shRNA at E13.5 and the harvested brain sections were immune-stained with mitotic marker pH3 at E16.5. Scale bar represents 50 μm.
F Graph shows the percentage of $pH3^+GFP^+$ cells in VZ/SVZ. The increase in the number of $pH3^+$ cells in the coronal sections of mH2A1.2 knockdown mice at E16.5. $n = 6$ mice, independent replicates.
G BrdU was injected intraperitoneally for 2 h of pulse labeling, and the harvested brain sections were immune-stained with anti-BrdU. Scale bar represents 50 μm. $n = 8$ mice, independent replicates.
H Percentage of GFP^+BrdU^+ cells among GFP^+ cells in VZ/SVZ. More GFP^+ $BrdU^+$ cells were found in the brains of KO than in those of WT mice. $n = 8$ mice, independent replicates.

Date information: Representative images from at least three independent experiments. Error bars represent means ± S.E.M.; two-tailed unpaired *t*-test, $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$. n.s., not significant.

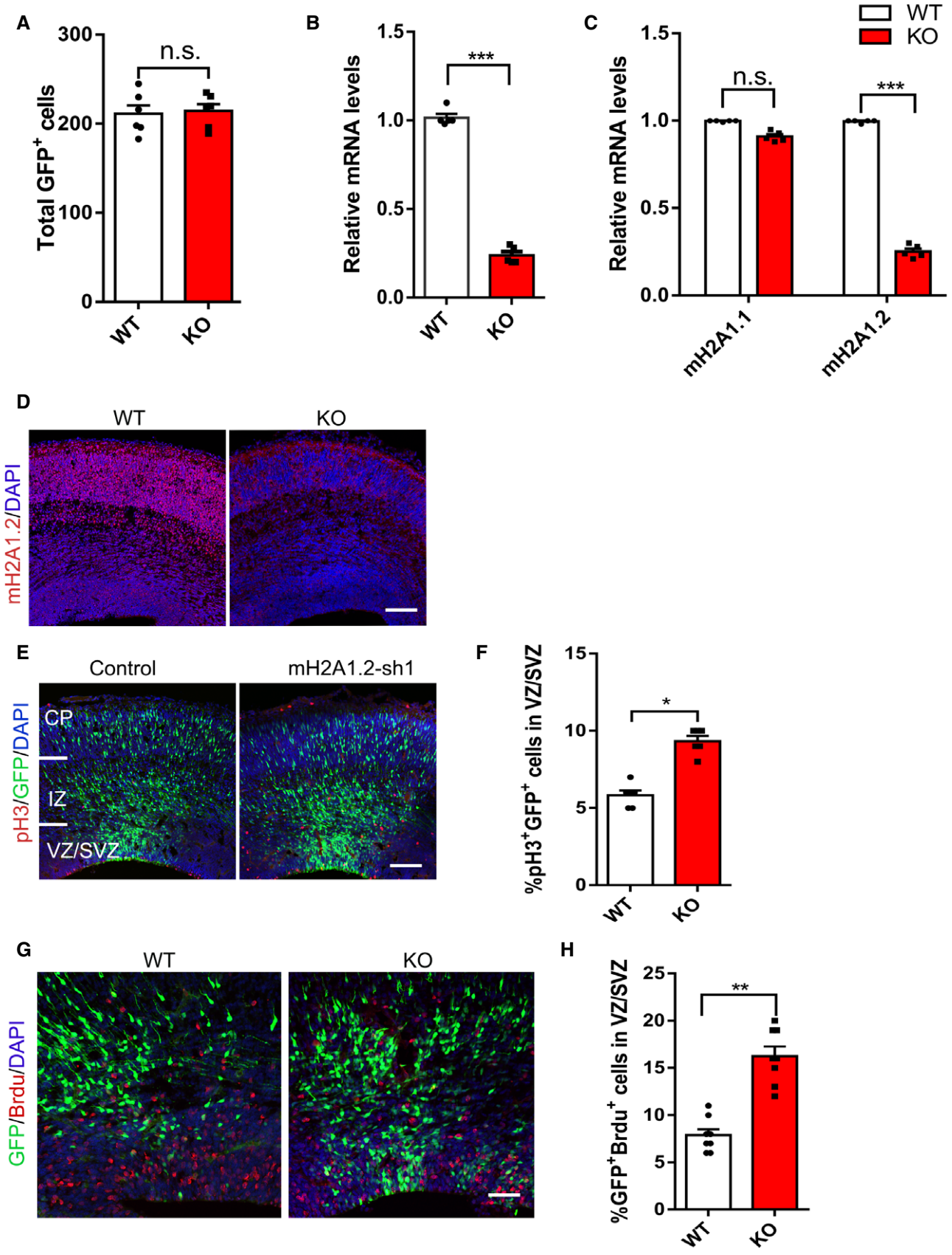
A
Total GFP+ cells
300
200
100
0
n.s.
WT
KO
B
Relative mRNA levels
1.5
1.0
0.5
0.0
***
WT
KO
C
WT
KO
Relative mRNA levels
1.5
1.0
0.5
0.0
n.s.
***
mH2A1.1
mH2A1.2
D
WT
KO
mH2A1.2/DAPI
E
Control
mH2A1.2-sh1
pH3/GFP/DAPI
CP
IZ
VZ/SVZ
F
%pH3+GFP+ cells in VZ/SVZ
15
10
5
0
*
WT
KO
G
WT
KO
GFP/Brdu/DAPI
H
%GFP+Brdu+ cells in VZ/SVZ
25
20
15
10
5
0
**
WT
KO

Figure EV2.

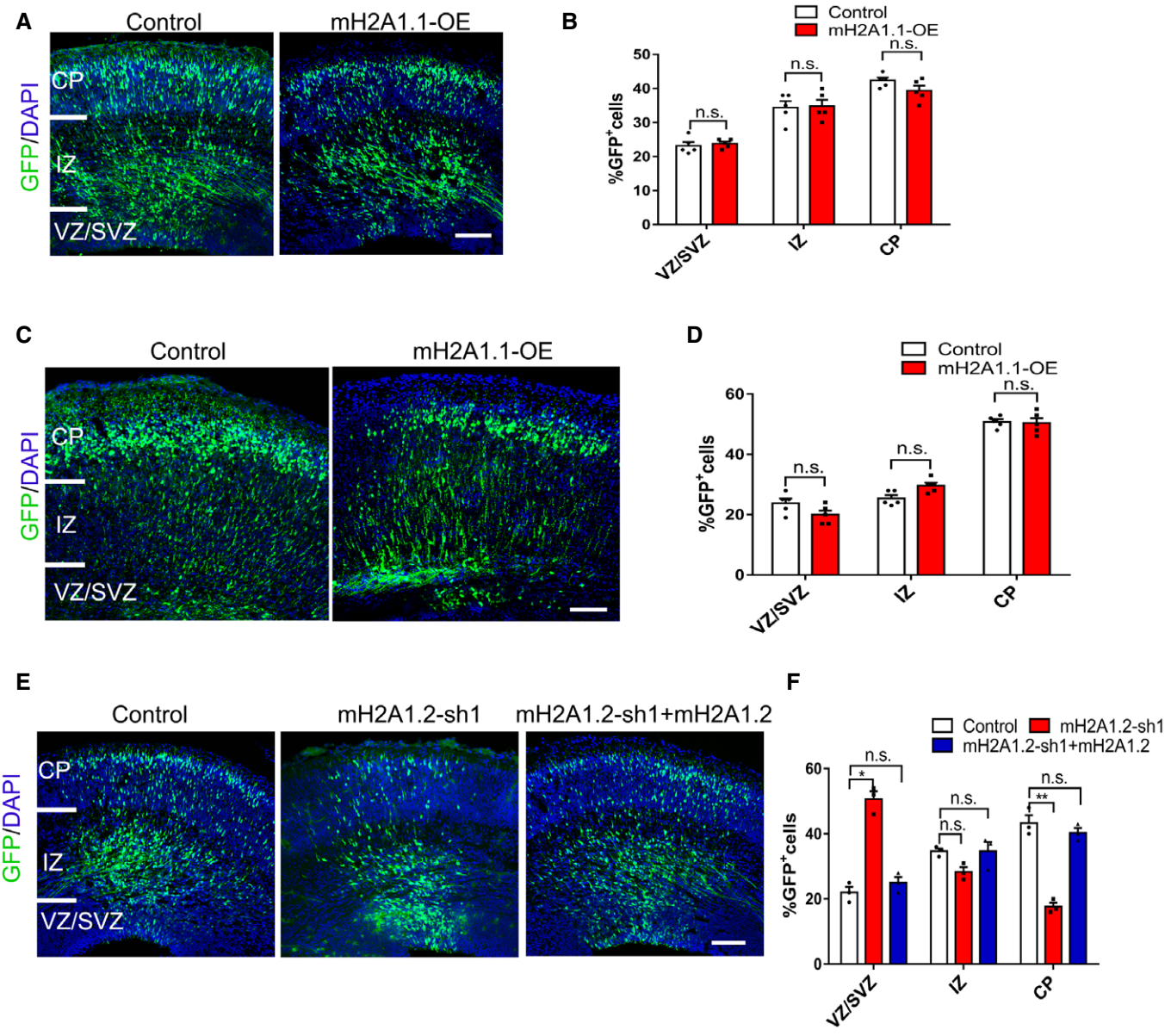

Figure EV3. The role of mH2A1.1 in neural stem cell differentiation and migration.

A The mH2A1.1 overexpressed plasmid was electroporated into mice brains at E13.5, and the mice were sacrificed at E16.5. Scale bar represents 50 μm.

B Graphs of the percentage of GFP-positive cells in the VZ/SVZ, IZ, and CP. The GFP-positive cells distribution no significant change. $n = 5$ mice, independent replicates.

C The mH2A1.1 overexpressed plasmid was electroporated into mice brains at E15.5, and the mice were sacrificed at P0. Scale bar represents 50 μm.

D Graphs of the percentage of GFP-positive cells in the VZ/SVZ, IZ, and CP. The GFP-positive cell distribution no significant change. $n = 5$ mice, independent replicates.

E Representative images of E16.5 cortices electroporated with Control, mH2A1.2-shRNA and mH2A-shRNA$^+$ mH2A1.2 into mice at E13.5, the mouse was sacrificed at E16.5. Scale bar represents 50 μm.

F Graphs of the percentage of GFP$^+$ cells in the VZ/SVZ, IZ, and CP. mH2A1.2-shRNA$^+$ mH2A1.2 restored the distribution of GFP$^+$ cells to the normal state. $n = 5$ mice, independent replicates.

Date information: Representative images from at least three independent experiments. Error bars represent means ± S.E.M.; two-tailed unpaired *t*-test, $^*P < 0.05$, $^{**}P < 0.01$. n.s., not significant.

Figure EV4. mH2A1.2 knockdown affects proliferation and differentiation of neural progenitor cells.

A Representative images of E16.5 cortices electroporated with GFP into WT and KO brain and GFP+mH2A1.2 into mice at E13.5, the mouse was sacrificed at E16.5. Scale bar represents 50 μm.

B Graphs of the percentage of GFP^+ cells in the VZ/SVZ, IZ, and CP. Overexpression of mH2A1.2 restored the distribution of GFP^+ cells to the normal state. $n = 5$ mice, independent replicates.

C Western blot analysis reveals that the expression levels of the neural stem cell markers, including pH3, PCNA, PAX6, TBR2, and mH2A1.2, are increased in the mH2A knockdown NSCs versus the control.

D Statistics of the normalized density of pH3, PCNA, PAX6, TBR2, and mH2A1.2. $n = 3$ mice, independent replicates.

E Western blot analysis reveals that the expression levels of the neuron markers NeuN, Tuj1, and mH2A1.2 are downregulated in mH2A1.2 knockdown NSCs versus the control.

F Statistics of the normalized density of NeuN, TUJ1, and mH2A1.2. $n = 3$ mice, independent replicates.

G Representative images of mH2A1.2 WT and KO cortex at newborn (P0) by hematoxylin and eosin stain (H&E) staining. Reduced cortical thickness is observed in KO cortex. CP: cortical plate. Scale bar = 800 μm.

H Quantification of the cortical thickness in mH2A1.2 WT and KO cortex. Reduced cortical thickness is observed in KO cortex. $n = 5$ mice, independent replicates.

Date information: Representative images from at least three independent experiments. Error bars represent means ± S.E.M.; two-tailed unpaired *t*-test, $*P < 0.05$, $**P < 0.01$. n.s., not significant.

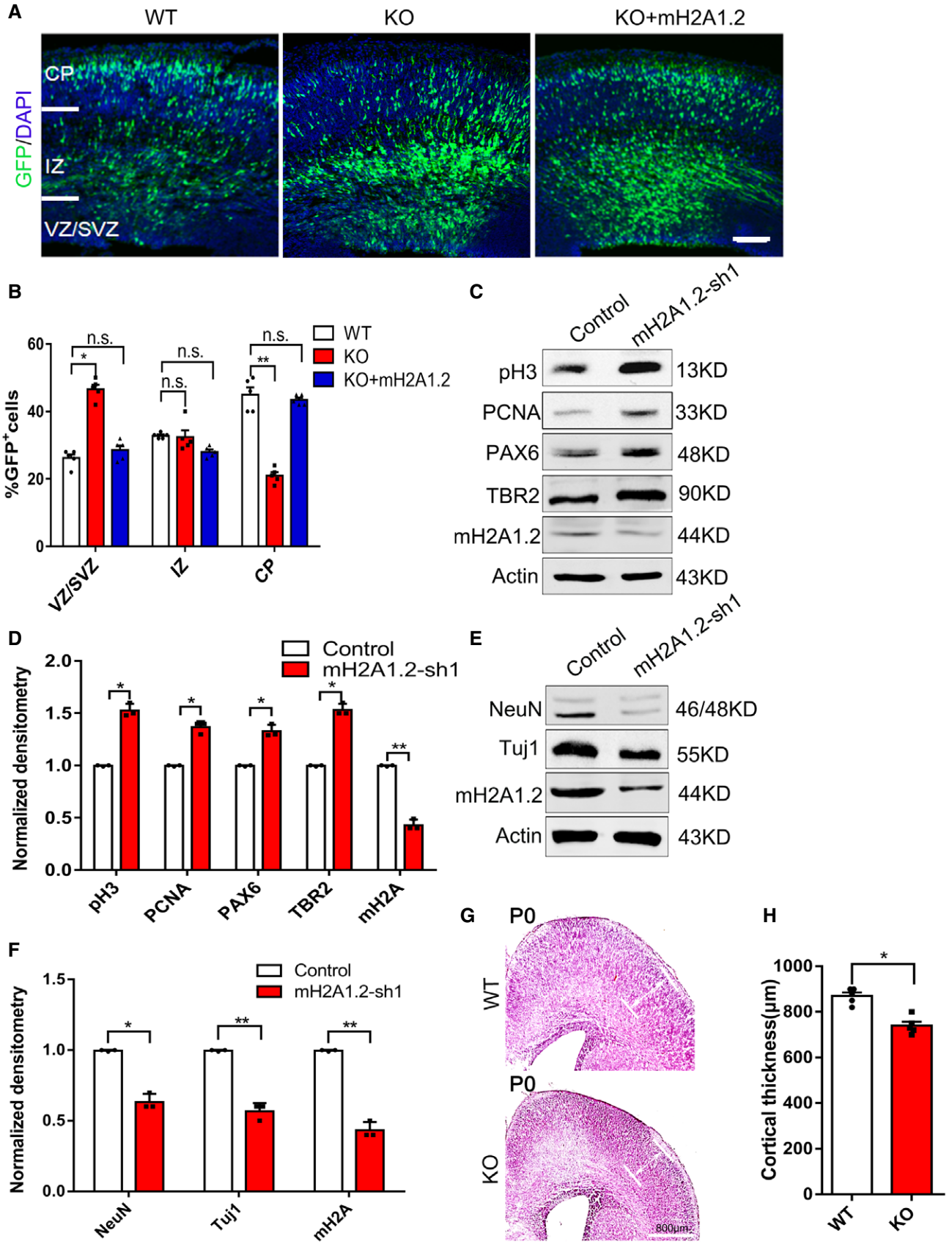

Figure EV4.

Figure EV5. mH2A1.2 knockout affects morphology of upper layer neurons and individual development.

A High-magnification confocal images of the upper layer show that mH2A1.2 KO results in abnormally branched processes compared with WT. The GFP plasmid was electroporated into the WT and KO embryonic brains at E13.5 and harvested at P7. Scale bar represents 10 μm.
B Regional amplification of upper layer neurons.
C High-magnification confocal images of the upper layer show that mH2A1.2 KO results in abnormally branched processes compared with WT. The GFP plasmid was electroporated into the WT and KO embryonic brains at E13.5 and harvested at P15. Scale bar represents 10 μm.
D Regional amplification of deep layer neurons.
E Quantification of dendritic numbers in WT and KO mouse cortices. $n = 5$ mice, independent replicates.
F Quantification of dendritic numbers in WT and KO mouse cortices. $n = 6$ mice, independent replicates.
G The brain sections were immune-stained with S100β at P7. Scale bar represents 20 μm.
H Graph shows the percentage of S100β cells in VZ/SVZ. $n = 5$ mice, independent replicates.
I Survival curves of WT and mH2A1.2 KO littermates, $n = 20$–30 for all samples.
J Images of the brain size and body size of WT and KO littermates at P0, P8, 5 weeks. Scale bar from left to right: 10 mm, 10 mm, 1 cm, 2 cm.

Date information: Representative images from at least three independent experiments. Error bars represent means ± S.E.M.; two-tailed unpaired *t*-test, $*P < 0.05$, $**P < 0.01$. n.s., not significant.

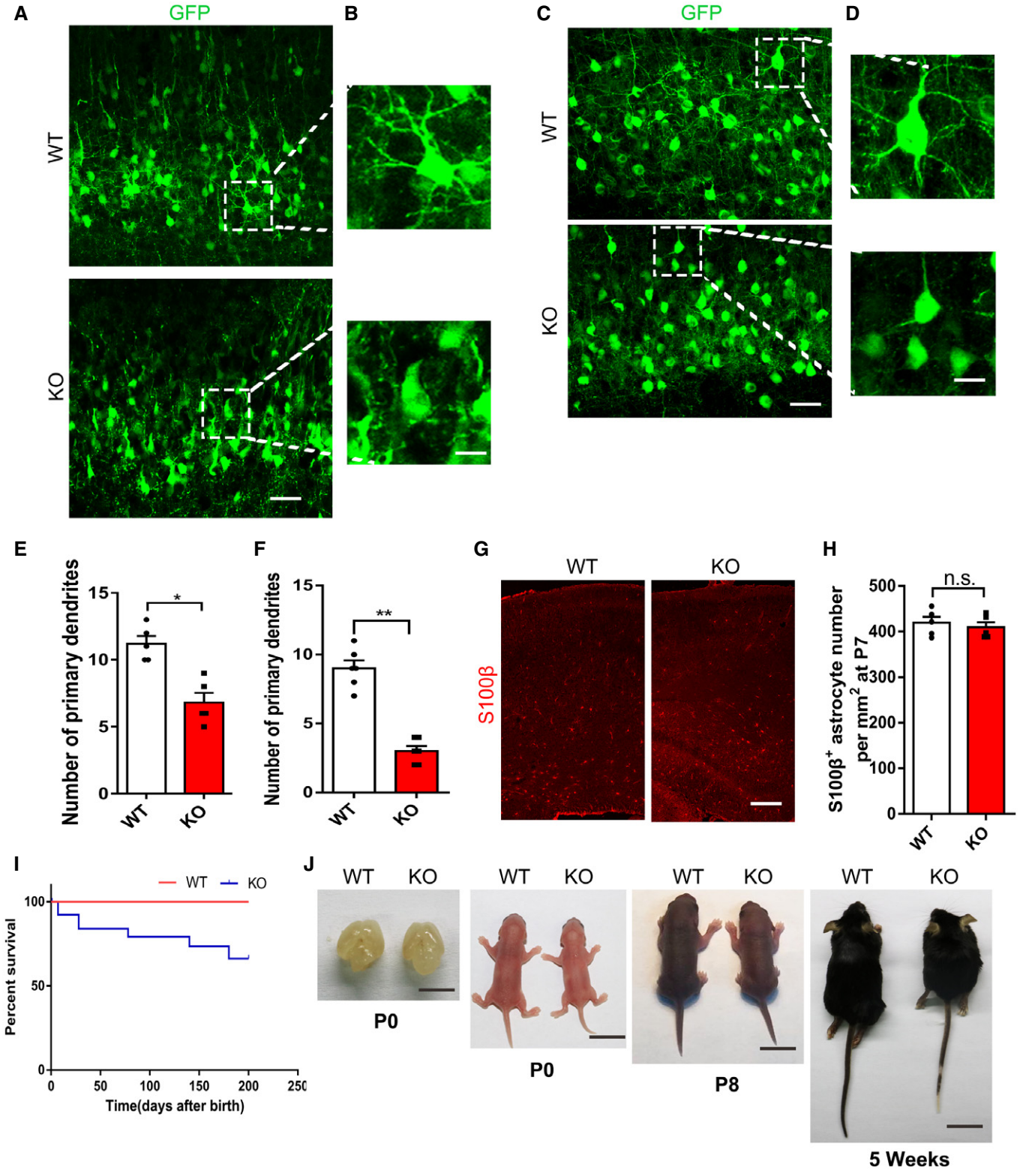

Figure EV5.